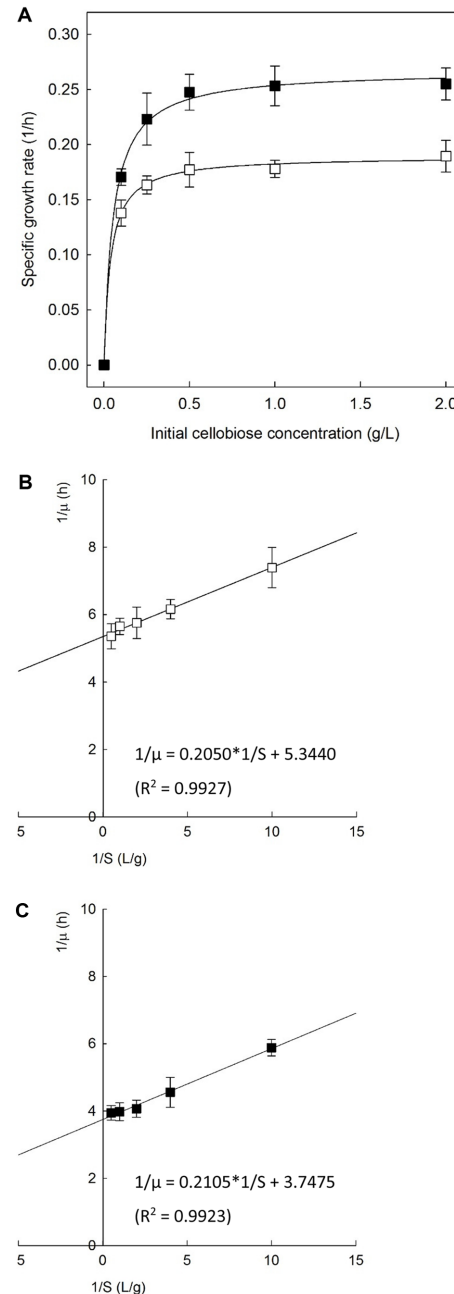


## Supplementary Materials

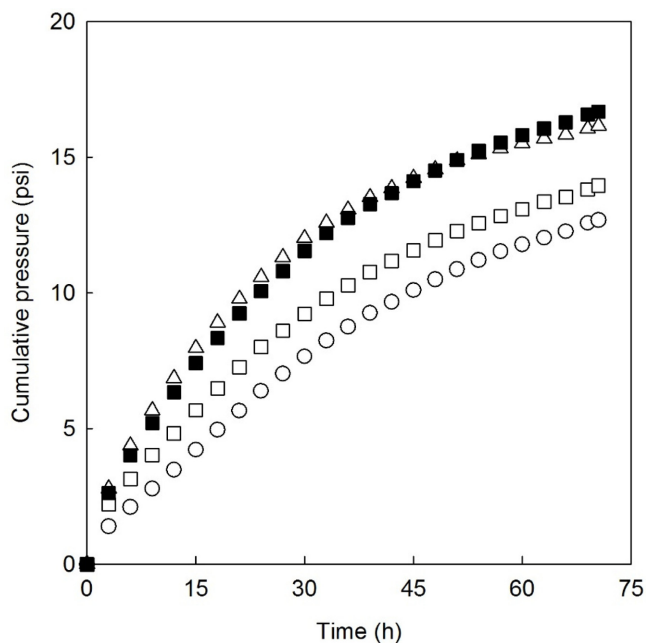
### Growth Kinetic Parameters of Cellobiose-Fermenting *S. cerevisiae* with the Phosphorolytic Pathway

To determine growth kinetic parameters of the phosphorolytic cellobiose-fermenting *S. cerevisiae* expressing SdCBP with either CDT-1 or CDT-1 (F213L) under cellobiose conditions, yeast cells at exponential growth in pre-cultivation were harvested and inoculated into 50 ml minimal (SC) medium (pH 6.0) with 0 to 2 g/l of cellobiose at an initial OD<sub>600</sub> of 0.05 [17]. The culture was carried out in 250 ml flasks at 30°C and 100 rpm. The OD values of the culture broth were checked every 2 h and were used to determine the Monod constant ( $K_S$ ) and the maximum specific growth rate ( $\mu_{max}$ ). All experiments were performed in triplicate.

As shown in Fig. S1, the  $K_S$  and  $\mu_{max}$  values were calculated using equations from the linear regression of the Lineweaver-Burk plots for the specific growth rates and different concentrations of the initial cellobiose (y-intercept =  $1/\mu_{max}$ , slope =  $K_S/\mu_{max}$ ). The  $\mu_{max}$  and  $K_S$  values of D-CTw (*S. cerevisiae* expressing wild-type CDT-1 and SdCBP) were determined to be  $0.19\text{ h}^{-1}$  and  $0.04\text{ g cellobiose/l}$ , respectively. The  $\mu_{max}$  and  $K_S$  values of D-CTm [*S. cerevisiae* expressing mutant CDT-1 (F213L) and SdCBP] were determined to be  $0.27\text{ h}^{-1}$  and  $0.06\text{ g cellobiose/l}$ , respectively. The growth kinetic parameters for the phosphorolytic *S. cerevisiae* strains were similar to those for the hydrolytic *S. cerevisiae* strains, D-BTw (*S. cerevisiae* expressing wild-type CDT-1 and GH1-1) and D-BTm [*S. cerevisiae* expressing mutant CDT-1 (F213L) and GH1-1], obtained from the previous study ( $0.04\text{ g cellobiose/g}$  and  $0.17\text{ h}^{-1}$  for D-BTw,  $0.06\text{ g cellobiose/l}$  and  $0.25\text{ h}^{-1}$  for D-BTm) [17].

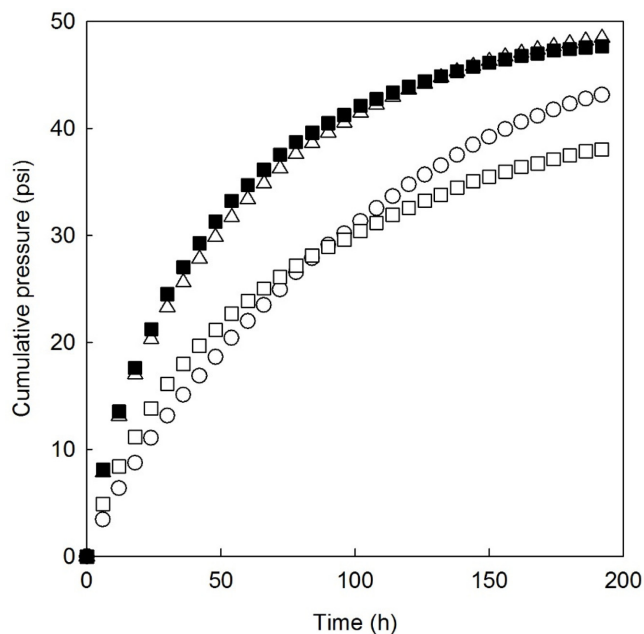


**Fig. S1.** Comparison of specific growth rates of the phosphorolytic cellobiose fermenting *S. cerevisiae* strains, D-CTw (the phosphorolytic *S. cerevisiae* expressing wild-type CDT-1; open square) and D-CTm (the phosphorolytic *S. cerevisiae* expressing mutant CDT-1; closed square), grown in minimal (SC) medium with different initial cellobiose concentrations (A). Lineweaver-Burk plots for the specific growth rates of D-CTw (B) and D-CTm (C) over initial cellobiose concentrations.



**Fig. S2.** Comparison of CO<sub>2</sub> production profiles in the anoxic SSF of 10% washed PCS with various cellobiose fermenting *S. cerevisiae* strains.

The gas production profiles published in the previous study [17] were compared with the gas profiles obtained in the current study. The yeast strains are denoted as follows: D-56+188 (the parental *S. cerevisiae* with extracellular  $\beta$ -glucosidase; open circle), D-BTm (the hydrolytic *S. cerevisiae* expressing mutant CDT-1; open triangle), D-CTw (the phosphorolytic *S. cerevisiae* expressing wild-type CDT-1; open square) and D-CTm (the phosphorolytic *S. cerevisiae* expressing mutant CDT-1; closed square). For SSF by D-56+188, Novozyme 188 (5.4 CBU/g glucan) as well as Celluclast 1.5L (10 FPU/g glucan) was used in the previous study.



**Fig. S3.** Comparison of CO<sub>2</sub> production profiles in the anoxic SSF of 13% Avicel PH-101 with various cellobiose-fermenting *S. cerevisiae* strains.

The gas production profiles published in the previous study [17] were compared with the gas profiles obtained in the current study. The yeast strains are denoted as follows: D-56+188 (the parental *S. cerevisiae* with extracellular  $\beta$ -glucosidase; open circle), D-BTm (the hydrolytic *S. cerevisiae* expressing mutant CDT-1; open triangle), D-CTw (the phosphorolytic *S. cerevisiae* expressing wild-type CDT-1; open square) and D-CTm (the phosphorolytic *S. cerevisiae* expressing mutant CDT-1; closed square). For SSF by D-56+188, Novozyme 188 (5.4 CBU/g cellulose) as well as Celluclast 1.5L (10 FPU/g cellulose) was used in the previous study.